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14. ABSTRACT

Dengue virus (DENV) is a major threat to military service members and global health. 5-20% of symptomatic patients progress to severe dengue (SD), manifested by complications and sometimes death, however, there are no accurate means to predict which patients will progress to SD. There is thus a critical need for biomarkers to effectively predict the development of severe complications and allow adequate patient triage. The goals of this project are to profile the host response to natural dengue infection in multiple cell subtypes in order to identify candidate biomarkers of dengue severity and novel targets for host-targeted anti-DENV agents; and ii) determine the feasibility for predicting SD by our novel gene set. To achieve these goals, we have been monitoring the host response to natural dengue infection in blood samples from the Colombia cohort using our novel platform and advanced immune monitoring technologies. In parallel, we have been validating the 20-gene set predictive of SD in a larger scale and identified a more parsimonious 8-gene set with a comparable predictive power.

15. SUBJECT TERMS
Antivirals, kinase inhibitors, dengue virus, Ebola virus, broad-spectrum antivirals, host-targeted antivirals

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1. INTRODUCTION:

Dengue virus (DENV), the FY18 PRMRP Topic Area this project addresses, represents a major threat to military service members and global health. Dengue has substantially weakened US military operations since the Spanish–American War and continues to represent a threat to military troops. Dengue infection is estimated to affect up to 400 million people annually in over 100 endemic countries and has become a leading cause of morbidity and mortality. With this global increase in dengue incidence and severity, a larger number of cases is already being reported among the active-duty personnel of the US Department of Defense and is expected to continue to increase. 5-20% of symptomatic patients progress to severe dengue (SD), manifested by complications and sometimes death. Early administration of supportive care reduces mortality, however, there are no accurate means to predict which patients will progress to SD. There is thus a critical need for biomarkers to effectively predict the development of severe complications and allow adequate patient triage. Moreover, there is a critical need for drugs and effective vaccines to combat dengue and/or prevent it.

Our overall goal is to understand the virus-host interplay involved in the development of SD more deeply and advance the development of both prognostic tools for early identification of patients at risk for progression to SD and antiviral strategies to prevent SD. We established a unique cohort in Colombia--dengue patients who present prior to progressing to SD. Moreover, we developed a novel platform, which transforms our ability to monitor the host response to dengue in thousands of individual cells. Additionally, we used a novel bioinformatics analysis of the publicly available gene expression data sets to identify a 20-gene set predictive of SD. Lastly, we demonstrated a proof-of-concept for the utility of targeting host factors (rather than viral factors) as an approach to combat DENV.

The goals of this project are to profile the host response to natural dengue infection in multiple cell subtypes in order to identify candidate biomarkers of dengue severity and novel targets for host-targeted anti-DENV agents; and ii) determine the feasibility for predicting SD by the novel 20-gene set. To achieve these goals, we will monitor the host response to natural dengue infection in blood samples from the Colombia cohort using our novel platform and advanced immune monitoring technologies. The functional relevance of prioritized biomarker and druggable host target candidates emerging from these studies will be probed and their roles in the development of SD and the DENV life cycle will be deciphered. In parallel, we will validate the 20-gene set predictive of SD in a recently published cohort and in the Colombia dengue cohort, monitor its dynamic during the disease course, and define its specificity.

1. KEYWORDS:

Dengue virus, severe dengue, pathogenesis, biomarkers, virus-host interactions, single-cell transcriptomics, immune monitoring

2. ACCOMPLISHMENTS:

The major goals of the project and the accomplishments under these goals

Aim 1. Map an atlas of DENV immune cellular targets and identify biomarkers of severity and candidate druggable host functions for antiviral therapy via single-cell gene expression and immune response profiling in natural infection.

We will define subpopulations of DENV-infected cells with high granularity by viscRNA-seq and CyTOF analysis in PBMC samples obtained at the peak of viremia from dengue patients from the Colombia cohort. We will also use these technologies to comprehensively monitor gene expression, virus genomics, and immune responses in longitudinal PBMC samples from the Colombia cohort to identify candidate biomarkers predictive of or associated with SD and host factors whose expression correlates with cellular virus abundance. The functional relevance of prioritized biomarker and druggable host target candidates

emerging from these studies will be probed and their roles in SD pathogenesis and the DENV life cycle will be deciphered.

Preliminary data for Aim 1

Development of viscRNA-seq platform to study host response and viral RNA (vRNA) abundance in single cells with an unprecedented resolution[1]. This platform enabled, for the first time in systems virology, to correlate host gene expression dynamics with abundance of any virus (not just polyadenylated) in thousands of single cells. We revealed a large heterogeneity in the host response to DENV and pro- and antiviral factors[1].

viscRNA-seq monitors immune responses to natural dengue infection in distinct cell populations (**Fig. 1**)[2]. We previously reported a pilot study in adults from our cohort[2]. With support from the Catalyst award, we recently studied samples from 20 patients with dengue (D, n=4), dengue with warning signs (DWS, n=4), patients who progressed to SD (n=8), and healthy controls (n=4). To increase breadth, we furthered the technology by combining our FACS-assisted visc protocol with the 5' 10X Genomics platform that profiles 10^3-10^4 cells per sample (vs. 10^2-10^3 via SMARseq2)[3].Cell staining followed by FACS sorting enabled capture of multiple immune cell populations (**Fig. 2 and Fig. 2 in [2]**). Our data analysis indicate that distinct cell populations respond differently to DENV infection (data not shown).

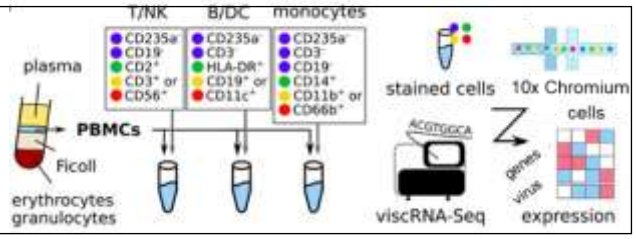


Fig. 1. Multi-faceted FACS-assisted viscRNA-Seq/10x analysis on PBMCs from dengue patients. PBMCs from the Colombia cohort patients are isolated via Ficoll centrifugation and stained with antibody panels to isolate different cell types. After balancing cell populations, single cells are loaded onto a 10X chromium chip, where they are partitioned with gel beads, RT enzymes, and our specific vRNA probe into droplets to simultaneously quantify single cell virus abundance and host transcriptome changes. Each cell is sequenced at a depth of ~10^5 reads on NovaSeq 6000 (illumina).

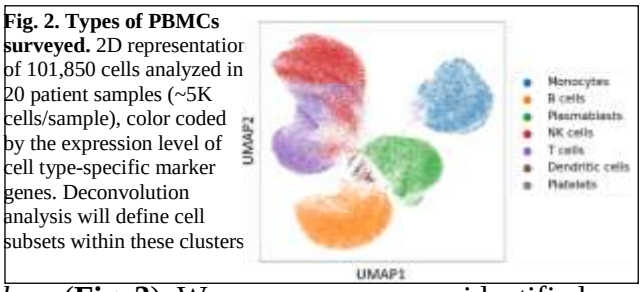


Fig. 2. Types of PBMCs surveyed. 2D representation of 101,850 cells analyzed in 20 patient samples (~5K cells/sample), color coded by the expression level of cell type-specific marker genes. Deconvolution analysis will define cell subsets within these clusters

Discovery of cell-type specific candidate predictive biomarkers (**Fig. 3**). We identified genes whose expression was dramatically altered prior to the onset of SD in distinct cell populations and thus represent candidate predictive biomarkers[2]. To assess their predictive power, we averaged their expression across cells within specific cell subtypes in samples obtained prior to progressing to SD to those from uncomplicated dengue patients and plotted the data by receiver operating characteristic (ROC) curves at increasing discriminatory thresholds for gene expression vs. disease severity. Most promising in adults (and children) are TMEM176B, SELL and CD163 in monocytes and CCND2 in plasmablasts, with an area under the ROC curve (AUCROC) of 0.91-1.

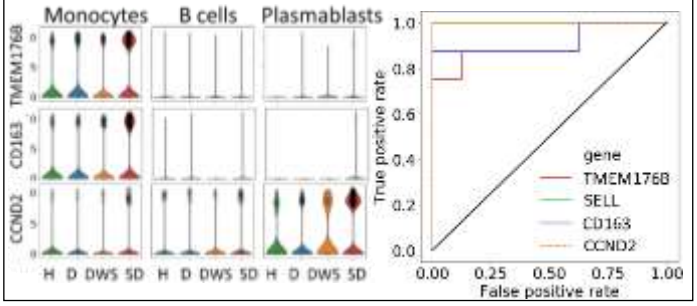


Fig. 3. Discovery of cell type-specific candidate predictive biomarkers for SD via viscRNA-seq. Left panel: Expression of TMEM176B and CD163 in monocytes and of CCND2 in plasmablasts is dramatically increased several days before severe symptoms in children. Healthy (H); Dengue (D); Dengue with warning signs (DWS); and Severe dengue (SD). Right panel: ROC curves of TMEM176B, SELL and CD163 expression in monocytes and of CCND2 in plasmablasts in children who progressed to SD vs. those with uncomplicated dengue. (The lines of SELL and CCND2 overlap).

A pilot CyTOF study reveals alterations in cell type abundance and responses prior to SD. We have used CyTOF, a proteomic single cell approach, to better understand SD pathogenesis.

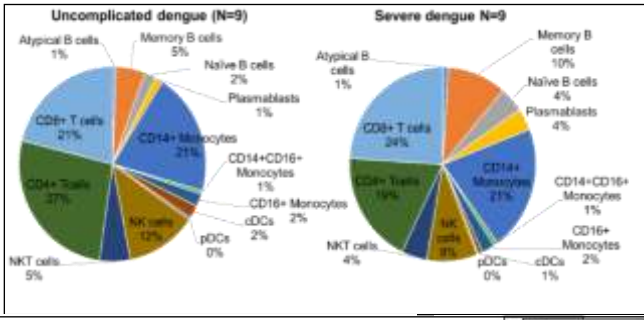
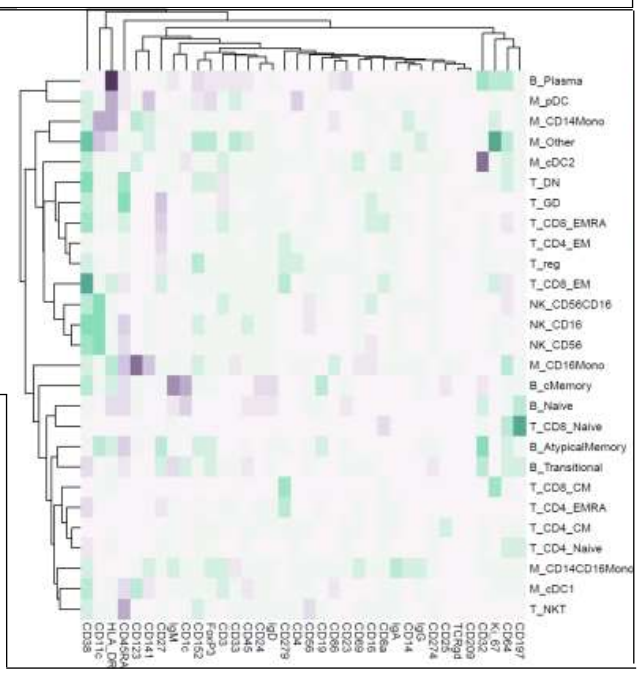


Fig. 4. **Quantification of immune cell populations via a pilot CyTOF analysis**. Data from a pilot adult study in samples from 9 patients with uncomplicated course and 9 patients who ultimately progressed to SD is shown. These Venn diagrams show a prominent expansion of B cell populations and mild alterations in other lineages (such as reduction in NK cells) prior to progression to SD.

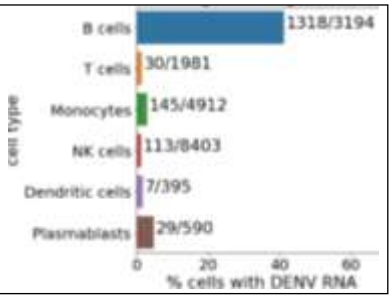
Fig. 5. Heat map of a pilot CyTOF experiment data showing differential gene expression in distinct cell populations between dengue and SD patients. Shown are median expression values of proteins (indicated on the bottom) in multiple distinct cell subtypes (indicated on the right). Data was derived from 9 adult patients who ultimately progressed to severe dengue (SD) and 9 with an uncomplicated dengue course (D). Differentially expressed proteins are color coded according to the scheme (purple, underexpressed in SD relative to D; green, overexpressed in SD relative to D). In face of the small sample size, several exciting patterns are observed, such as increased CD32B (inhibitory FcγR) and decreased HLA-DR (activation marker) expression in distinct subpopulations of B cells in SD suggesting that they may have impaired responses; increased CD11c and CD38 expression in NK populations supporting their activation in SD; and increased PD1 (CD279) ex



We assembled an antibody panel that is enriched for B cell and monocyte markers (based on the viscRNA-seq data) and includes antibodies targeting three DENV proteins. When studying ~30 PBMC samples from our cohort, this panel identified altered abundance of distinct cell subtypes (**Fig. 4**) and differentially expressed proteins in SD vs. dengue (**Fig. 5**).

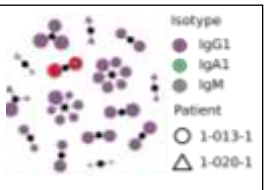
Identifying the target cells of DENV in the human blood. Optimization of our DENV specific probe enabled capture of vRNA harboring cells via viscRNA-seq in 10 of 16 DENV-infected patients (vs. 2 of 8 in our pilot). Most of these cells are naïve B cells (as in adults), yet vRNA is also detected in plasmablasts, monocytes and other cell types (**Fig. 6**). These cells respond differently to DENV infection vs. bystander cells (data not shown). CyTOF detected DENV+ cells comprising ~2-5% of the total PBMCs, consistent with previous reports[4]. Interestingly, the vRNA-associated B cells discovered via viscRNA-seq do not seem to harbor intracellular viral proteins, and thus may have a different role (e.g. viral spread to secondary tissues) rather than supporting viral replication. In contrast, monocytes appear to be associated with vRNA and to harbor viral proteins intracellularly, suggesting that these are targets of viral replication.

Fig. 6. Naïve B cells followed by plasmablasts and monocytes harbor DENV RNA in dengue patients. Shown are the fraction of cells containing vRNA across cell types from three children with high DENV viremia.



Informing the design of a dengue vaccine[2, 5] (**Figs. 7-8**). The development of a safe, effective vaccine for DENV is challenged by the need to elicit antibodies that neutralize all four viral serotypes simultaneously to minimize ADE risk[6, 7]. We leveraged our viscRNA-seq data of plasmablasts from acute secondary DENV infection and screened clonally expanded and somatically hypermutated B cells[2] (likely to encode antigen-specific, affinity matured

Fig. 7. BCR clonal families reconstructed from plasmablasts in DENV patients. Graph of heavy chain CDR3 antibody clonality showing clonal expansion of IgG1 plasmablasts in patients 1-013-1 and 1-020-1. Each dot is a unique antibody sequence; larger size corresponds to more somatic hypermutation. Large clonal families comprised of multiple plasmablasts sharing similar antibody heavy chains, indicating a rapid and large clonal expansion in the B cell compartment is shown.



antibodies). We identified two clonally related broadly neutralizing antibodies (bNAbs), J8 and J9, that neutralized DENV1-4 in a low pM range and recognized a determinant in the DENV E protein, distinct from previously characterized bNAbs. Analysis of the corresponding B cell repertoire revealed divergent evolution, suggesting multiple evolutionary pathways to generate bNAbs within this lineage[2, 5]. This discovery is exciting, as it indicates that developing a conserved epitope-based vaccine strategy to elicit bNAbs could mitigate the challenge of selecting representative vaccine strains.

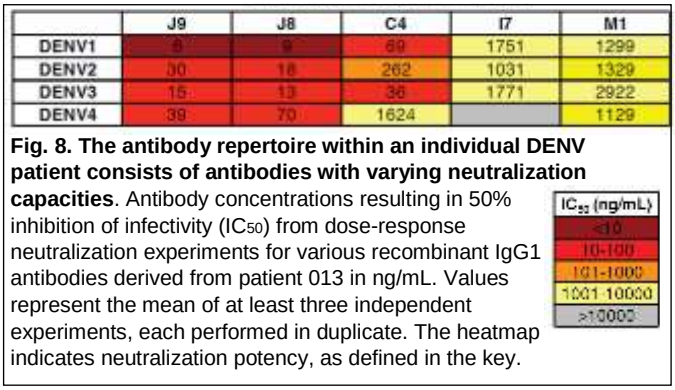


Fig. 8. The antibody repertoire within an individual DENV patient consists of antibodies with varying neutralization capacities. Antibody concentrations resulting in 50% inhibition of infectivity (IC_{50}) from dose-response neutralization experiments for various recombinant IgG1 antibodies derived from patient 013 in ng/mL. Values represent the mean of at least three independent experiments, each performed in duplicate. The heatmap indicates neutralization potency, as defined in the key.

Milestones achieved under Aim 1:

1. Enrollment to the dengue cohort exceeded the plan for year 1: we enrolled a total of 193 adults (vs. 139 originally proposed).
2. Distinct virus-associated cell subtypes detected in 10 samples from DENV-infected patients.
3. Subsets of DENV-associated cells defined via viscRNA-seq and CyTOF.
4. A pilot CyTOF assay on 30 patient samples conducted.
5. "Hits" with greatest prediction for SD defined; 6. A new class of potent bNAbs against dengue identified (not proposed originally).

Aim 2. Determine the feasibility and biological rationale for predicting severe dengue by the novel prognostic 20-gene set.

We will validate the 20-gene set predictive of SD in a recently published cohort and in the prospective Colombia dengue cohort, monitor its dynamic during the disease course, and define its specificity. Additionally, we will decipher the roles of some of the 20 gene products in SD pathogenesis and the DENV life cycle.

Preliminary data for Aim 2

A 20-gene set predictive of progression to SD. To capture real world heterogeneity, we used a multi-cohort analysis of publicly available gene expression data sets, developed by Dr. Khatri. We initially identified and *in silico* validated a 20-gene set predictive of SD[8]. We recently prospectively validated this 20-gene set in 124 x adults and 60 children (Catalyst) patients from the Colombia cohort[8] and demonstrated an excellent predictive power with an AUCROC of 0.914 (95% CI: 0.83-0.99) to distinguish between patients who progressed to SD and those with uncomplicated course (dengue or dengue with warning signs (DWS)) and of 0.902 (95% CI: 0.81-0.98) between SD and DWS (**Fig. 9**). These values are even better than the AUCs we previously measured in the original 10 published datasets and pilot prospective validation. Moreover, we have validated the 20-gene set in a recently published dataset from an independent Cambodia cohort composed of 35 dengue patients with an AUCROC of 0.75 (95% CI 0.56-0.97) for SD vs. D.

The 20-gene set appears specific for dengue. No overlap was observed between the dengue scores calculated in 6 Zika patients and 20 healthy controls to those in SD patients (data not shown).

The 20-gene set is triggered by dengue infection. The high dengue scores measured in SD patients declined over time in longitudinal samples collected during the disease course and after clinical recovery (data not shown), suggesting that this signature is triggered by DENV infection rather than precedes the infection.

Discovery and validation of an 8-gene set predictive of SD. While the 20-gene set is very promising, to facilitate development of a more cost-effective prognostic assay, we have pursued efforts to reduce the number of genes in the set. To that end, the Khatri lab has furthered its bioinformatics approach. The 11 clinical publicly available dengue datasets (N = 565) were randomly selected for Discovery (7) and validation (4) and subject to the multi-cohort analysis with the goal to distinguish between uncomplicated dengue and SD. Following 100 iterations of this procedure, 25 genes that passed thresholds in >50% iterations were identified. These genes are robust to dataset selected for discovery analysis, which in turn (1) increases their likelihood of being generalizable across a broad patient population and (2) decreases *a priori* risk of irreproducibility in a prospective cohort for validation. Applying a greedy forward search algorithm to optimize the AUC in the 11 datasets yielded an 8-gene signature that partially overlaps with the 20-gene set with a mean AUC of 0.85. Prospective validation of the 8-gene set in 184 dengue patients from the Colombia cohort revealed a predictive power that is comparable to the 20-gene set with an AUCROC of 0.899 (95% CI: 0.81-0.89) to distinguish SD from uncomplicated dengue and 0.888 (95% CI: 0.78-0.89) to distinguish between SD and DWS (**Fig. 9**).

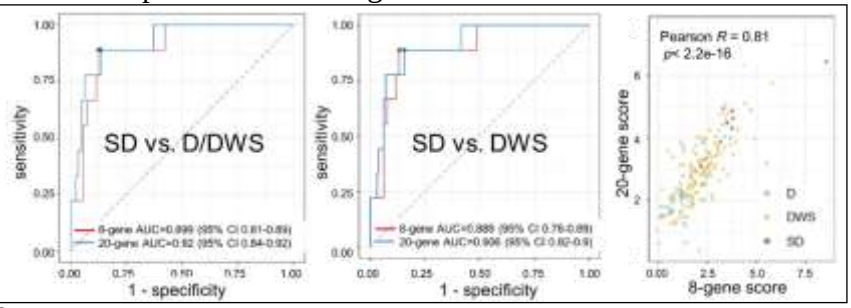


Fig. 9. Prospective validation of the 20- and 8-gene sets. Transcript levels in patient blood samples were measured by NanoString nCounter gene expression assay. ROC curves comparing SD vs. dengue with (DWS) or without (D) warning signs (left panel) or SD vs. DWS (mid panel) in 184 patients from the Colombia cohort. Right panel: Scatter plot of 20-gene vs. 8-gene scores showing good correlation in predicting progression to SD.

Milestones achieved under Aim 2: We have met all the milestones, and in fact have far exceeded them. 1. Predictive power of the 20-gene set demonstrated in a recently published Cambodian cohort; 2. Patient enrollment to the Colombia cohort has been on target (193 adults and 267 children (Catalyst)) and has enabled larger scale validation; 3. 20-gene set validated in 184 patients from our cohort; 4. A highly predictive, independent 8-gene set identified and validated in 184 patients from the Colombia cohort.

Training opportunities that the project provided

- Students and postdoctoral fellows at the Einav, Khatri and Pinsky labs have been mentored by the respective PI and benefited from interdisciplinary training provided by this project. The PIs were actively involved in designing experiments, data analysis, overseeing the study strategies and participating in presentations and manuscripts preparation.

- The trainees at Stanford enjoyed the privilege of participating in the Stanford Infection Transplantation and Immunology (ITI) seminars and meeting as well as in the Stanford SPARK program; a Stanford-based initiative aimed at advancing discoveries into the clinic. It is directed by Dr. Mochly-Rosen and egage a large number of consultants with expertise in multiple aspects of the development process including assay development, regulatory process, etc. The trainees meet on a weekly basis with this talented group of consultants. In addition, they presented their progress to the SPARK medicinal chemistry group every quarter.

- All the trainees on this project have also benefited from regular conference calls, which enriched their multidisciplinary training opportunities to students and research fellows.

- The Stanford trainees have attended the annual Bay Virology Symposium, the annual retreats of the Microbiolgy Department and the division of infectious diseases, as well as monthly seminars of the NIH U19 (to SE) which was focused on developing host-targeted broad-spectrum antiviral approaches. They also attended monthly seminars of the arboviral working group at Stanford, which is directed by Dr. Einav and integrates members from 16 labs on campus, all dedicated to study mosquito transmitted viruses.

Dissemination of the results to communities of interest
We presented this work in various local meetings and in an international meeting. We were also invited to write a perspective about biomarkers for dengue, which summarized others' and our work in the field.

What do you plan to do during the next reporting period to accomplish the goals?
1. We will expand our visc-RNA seq analysis, study longitudinal samples and compare this dataset with our pediatric data set (CCHI/Catalyst funded) to identify determinants that may explain the increased dengue severity in children.
2. We will conduct CyTOF analysis on additional adult samples (total: 60) and compare the data to a similar dataset generated in children (CCHI/Catalyst funded).
3. We will define the predictive potential of the identified factors at a larger scale and start probing their roles in SD pathogenesis.
4. We will start to phenotypically characterize the relevant cell populations that harbor vRNA.
5. We will reconstruct BCR clonal families from additional DENV patients and collaborate with Dr. Goo to identify and profile bNAbs.
6. We will prospectively validate the 20-gene set and 8-gene set in additional samples from the Colombia cohort.
7. We will start engaging companies to identify a platform suitable for a prognostic assay development.

4. IMPACT:

The impact on the development of the principal discipline(s) of the project
The predicted conceptual contribution is providing insights into the pathogenesis of SD at an unprecedented resolution. This project will also reveal cell type-specific predictive biomarkers and candidate targets for antivirals or vaccines. The translational impact is advancing the development of the first paradigm-shifting molecular assay to both diagnose dengue infection and predict SD prior to its onset. Such an assay can help define the level of patient care, thereby reducing morbidity and mortality while allocating resources more effectively, and guide the design of therapeutic clinical trials and future treatment decisions once anti-DENV therapies (as those we are developing) are approved. Moreover, identifying druggable host factors that are overexpressed in virally-infected but not bystander cells is innovative and attractive, as their inhibition may prevent SD and is more likely to be safe. This work may thus advance the development of host-targeted antiviral approaches to combat DENV and possibly other emerging viruses with a high genetic barrier to resistance, thereby diverting from the prevailing direct acting antiviral treatment paradigm. Lastly, identifying functional correlates of *in vivo* clearance can guide vaccine development, as we recently reported.

The impact on other disciplines
From a technological standpoint, our viscRNA-seq approach, including increased scalability via droplet microfluidics and multiplexing, aims to become a powerful approach to gain knowledge on any viral infection in humans at the single cell level.

Moreover, the improved multi-cohort analysis framework has transformed our ability to discover gene sets associated with disease outcome.

The impact on technology transfer
Nothing to Report.

The impact on society beyond science and technology
Nothing to Report.

5. CHANGES/PROBLEMS:
Changes in approach and reasons for change
There have been no changes in objectives or scope.

Actual or anticipated problems or delays and actions or plans to resolve them
None

Changes that had a significant impact on expenditures
None
Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
None
Significant changes in use or care of human subjects
N/A
Significant changes in use or care of vertebrate animals.
None
Significant changes in use of biohazards and/or select agents
None

6. PRODUCTS:

Journal publications:
1. Durham ND, Agrawal A, Waltari E, Croote D, Zanini F, Fouch M, Davidson E, Smith O, Carabajal E, Pak JE, Doranz BJ, Robinson M, Sanz AM, Albornoz LL, Rosso F, Einav S, Quake SR, McCutcheon KM, Goo L. Broadly neutralizing human antibodies against dengue virus identified by single B cell transcriptomics. eLife. 2019 Dec 10;8. pii: e52384. doi: 10.7554/eLife.52384. PMID:31820734

2. Robinson, M.L. and Einav, S. (2019). Towards predicting progression to severe dengue. Trends in Microbiology. 2020 Jan 22. pii: S0966-842X(19)30318-X. doi: 10.1016/j.tim.2019.12.003. PMID:31982232 (Invited Review)

Presentations:

"Capturing tissue and real-world heterogeneity to better understand the pathogenesis of severe dengue." University of Pennsylvania, Microbiology and Immunology seminar. Philadelphia, Oct 2019.

"Towards better understanding and predicting severe dengue: from single cell to multi-cohort transcriptomic analyses." Twincore 11th Symposium: "Infection research meets big data", Twincore, Hanover, Germany, Aug 2019.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Individuals who have worked on the project throughout the award period:

Name:	**Shirit Einav**
Project Role:	PD/PI
Researcher Identifier (e.g. ORCID ID):	0000-0001-6441-4171
Nearest person month worked:	3
Contribution to Project:	In charge of coordinating between the teams on the project, designing experiments, ensuring research goals are met in a timely manner and within budget, training the student and postdocs on this project etc.
Funding Support:	DoD, DTRA, NIH (pilot)
Name:	**Sirle Saul**
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	0000-0001-7845-9418
Nearest person month worked:	6
Contribution to Project:	Executes the experiments involving CyTOF and single cell analysis.
Funding Support:	DoD, DTRA
Name:	**Pinsky, Benjamin**
Project Role:	Co-Investigator

Researcher Identifier (e.g. ORCID ID):	0000-0001-8751-4810
Nearest person month worked:	0.12
Contribution to Project:	Confirm DENV diagnosis, measure viremia, define specific serotype, and study prior exposure to dengue.
Funding Support:	DoD, NIH
Name:	Huang, Chunhong (Stacey)
Project Role:	Life Science Researcher Prof. 2 (technician)
Researcher Identifier (e.g. ORCID ID):	0000-0002-3049-086X
Nearest person month worked:	1.6
Contribution to Project:	Assisted PI with the scope of work: Confirm DENV diagnosis, measure viremia, define specific sero dengue.
Funding Suppor:	DoD, NIH
Name:	Purvesh Khatri
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	0000-0002-4143-4708
Nearest person month worked:	1.15 CM

Contribution to Project:	Co-I Khatri will oversee the efforts focused on validating the transcriptomic signature predictive of severe dengue by the integrated multi-cohort analysis and systems immunology analysis of the data generated by the single cell transcriptomic platform. He will be helping with data analysis, and be an active participant in presentation of research findings and preparation of manuscripts.
Funding Support:	DoD, NIH, BWH, GATES, EMD Serono, SPARK Weston Havens Fdn, InterMountain Healthcare, Lyme Disease

Name:	**Alexander Skrenchuck**
Project Role:	System Admstr 3
Researcher Identifier (e.g. ORCID ID):	None
Nearest person month worked:	0.60 CM
Contribution to Project:	Alex provides systems administration and user support of research computing infrastructure for Khatri Lab. This includes procurement, deployment, maintenance, troubleshooting of hardware, storage, networking, operating systems, databases, applications, High Performance Computing Clusters, virtualization, cloud solutions and other scientific research and development computing infrastructure.
Funding Support:	DoD, GATES
Name:	**Hong Zheng**
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	0000-0002-8884-8211
Nearest person month worked:	12 CM
Contribution to Project:	Hong Zheng performed multi-cohort analysis of host immune response and identified conserved protective and detrimental modules associated with severity of viral infection (https://www.medrxiv.org/content/10.1101/2020.10.02.20205880v1). The identified modules are being tested in dengue patients.
Funding Support:	DoD
Name:	**Ananth Ganesan**
Project Role:	Grad student research assistant
Researcher Identifier (e.g. ORCID ID):	0000-0001-6242-4261
Nearest person month worked:	3.60 CM

Contribution to Project:	Ananth's expertise are in the areas of Machine Learning and Data Science, and their applications to Immunology. He worked on the development of a novel computational method for the analysis of drug compounds, with specific focus on drugs with high efficacy against dengue virus. Ananth has also developed and implemented tools to impute missing data, classify disease states from gene expression and protein levels, and interpret models to obtain new insights on biological mechanisms.
Funding Support:	DoD, GATES
Name:	**Mike Seda**
Project Role:	System Admstr
Researcher Identifier (e.g. ORCID ID):	None
Nearest person month worked:	0.60 CM
Contribution to Project:	Mike Seda provides systems administration and user support of research computing infrastructure for Khatri Lab. This includes procurement, deployment, maintenance, troubleshooting of hardware, storage, networking, operating systems, databases, applications, High Performance Computing Clusters, virtualization, cloud solutions and other scientific research and development computing infrastructure.
Funding Support:	DoD, GATES

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

Other organizations involved as partners:
Outside of Stanford, we have been collaborating with Dr. Leslie Goo at the Fred Hutchinson Cancer Research Center and Dr. Fabio Zanini at the University of New South Wales

Otherwise there is nothing to report.

8. SPECIAL REPORTING REQUIREMENTS
N/A

9. APPENDIX:

A. **References Cited:**

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